CLAIMS

What is claimed is:

1. A method for identifying conditions, compounds, or compositions for modifying a crystal or a calculus comprising calcium oxalate, the method comprising:

preparing seed crystals of calcium oxalate;

incubating the seed crystals in a supersaturated calcium oxalate medium comprising at least one of a plurality of components;

detecting a first a crystal property; and

identifying a component corresponding to a change in the crystal property.

2. The method of claim 1 further comprising replacing the supersaturated calcium oxalate medium comprising the at least one of the plurality of components with a supersaturated calcium oxalate medium without components; and detecting a second crystal property.

3. The method of claim 2 wherein the second crystal property is the absence of crystal growth.

4. The method of claims 2 or 3 further comprising identifying at least one component that irreversibly binds to calcium oxalate crystals using the detected crystal property.

5. The method of claim 1 further comprising:

preparing a solution comprising sodium oxalate;

preparing a solution comprising calcium chloride; and

mixing the solution of sodium oxalate and the solution of calcium chloride to obtain a calcium oxalate solution.

6. The method of claim 5 wherein the supersaturated calcium oxalate medium comprises the calcium oxalate solution.

7. The method of claim 6 further comprising incubating the supersaturated calcium oxalate medium to obtain seed crystals.

8. The method of claim 7 further comprising detecting the seed crystals by photographing birefringent spots.

9. The method of claim 7 further comprising imaging the crystals in a sample by photographing birefringment spots prior to incubation, one or more times during incubation, at the end of incubation, or any combination thereof; and creating a comparison of crystals in a sample over time.

10. The method of claim 9 further comprising estimating or determining a nucleation rate of crystals in the sample from the plot of crystals in the sample over time.

11. The method of claim 10 further comprising selecting at least one component corresponding to increase in the nucleation rate as a therapeutic for treating, preventing or managing a disease, condition, or disorder associated with calcium oxalate crystals or calculi.

12. The method of claim 10 further comprising selecting at least one component corresponding to decrease in the nucleation rate as a therapeutic for treating, preventing or managing a disease, condition, or disorder associated with calcium oxalate crystals or calculi.

13. The method of claims 11 or 12 wherein the disease, condition, or disorder associated with calcium oxalate crystals or calculi is nephrolithiasis, ureterolithiasis, hyperoxaluria, or oxalosis.

14. The method of claim 6 wherein the calcium oxalate solution further comprises the at least one of a plurality of components.

15. The method of claim 14 wherein the at least one of a plurality of components changes the pH of the supersaturated calcium oxalate medium.

16. The method of claim 14 wherein the at least one of a plurality of components prevents the calcium oxalate medium from being supersaturated.

17. The method of claim 5 wherein the solution comprising calcium chloride further comprises the at least one of a plurality of components.

18. The method of claim 17 wherein the solution comprising calcium chloride has a different pH than a solution comprising of calcium chloride.

19. The method of claims 1 or 6 further comprising:

illuminating crystals with polarized light;

photographing the illuminated crystals via a cross polarizer to detect birefringent crystals; and

detecting a plurality of crystal spots from a photograph of the illuminated crystals.

20. The method of claim 19 further comprising photographing the illuminated crystals prior to incubation, one or more times during incubation, at the end of incubation, or any combination thereof.

21. The method of claims 19 or 20 further comprising:

analyzing the photograph of the illuminated crystals to estimate a number and/or position of pixels corresponding to a crystal spot;

comparing the number and/or position of pixels to an initial number of pixels corresponding to the crystal spot to detect a change in the number of pixels; and estimating a change in crystal size or shape from the change in the number and/or position of pixels.

22. The methods of claim 21 further comprising selecting at least one component corresponding to reduction or no change in an average crystal size as a therapeutic for treating, preventing or managing a disease, condition, or disorder associated with calcium oxalate crystals or calculi.

23. The method of claim 21 wherein the change in crystal size is measured as a function of time from a change in the number of pixels comprising the same individual crystal (spot) after various lengths of incubations.

24. The method of claim 22 wherein the disease, condition, or disorder associated with calcium oxalate crystals or calculi is nephrolithiasis, ureterolithiasis, hyperoxaluria or oxalosis.

25. The method of claim 19 further comprising:

analyzing the photograph of the illuminated crystals to estimate a long and a short dimension; and

estimating a measure of a habit corresponding to the crystal spot from the long and short dimensions.

26. The method of claim 1 further comprising identifying the component as a marker indicating susceptibility to nephrolithiasis, wherein the component is a fraction derived from urine.

27. The method of claim 5 wherein the solution comprising sodium oxalate further comprises urine.

28. The method of claim 26 wherein the solution comprising sodium oxalate is prepared in urine selected from the group consisting of human urine, non-human urine, synthetic urine, normal urine, and urine from a subject at risk of nephrolithiasis.

29. The method of claim 26 wherein the supersaturated calcium oxalate medium comprises a fraction from the urine of a nephrolithiasis susceptible subject.

30. The method of claim 1 further comprising estimating a composition of a crystal.

31. The method of claim 30 wherein Raman spectroscopy is used to estimate the composition of the crystal.

32. The method of claim 1 further comprising culturing kidney epithelial cells.

33. The method of claim 32 wherein the cultured cells are selected from the group consisting of MDCK, LLC-PK_1, mIMCD, and tubule fragments.

34. The method of claim 32 further comprising preparing labeled calcium oxalate crystals.

35. The method of claim 32 further comprising incubating labeled calcium oxalate crystals with cultured kidney epithelial cells.

36. The method of claim 35 further comprising solubilizing washed cells following incubation with labeled calcium oxalate crystals; and measuring a signal corresponding to a label in the labeled calcium oxalate crystals.

37. A method to identify conditions, compounds or compositions that promote modification of a calcium oxalate crystal or calculus, comprising:

preparing an array comprising at least 24 samples each sample comprising a medium and a calcium oxalate calculus or crystal;

processing one or more of the samples to induce the modification of the calcium oxalate calculus or crystal;

screening the array by analyzing the processed samples to detect the modification of the calcium oxalate calculus or crystal; and

selecting the samples wherein the modification of the calculus or crystals occurred to identify the conditions, compounds, or compositions of potential therapeutic value.

38. The method of claim 37, wherein the medium is synthetic, natural, or semi-synthetic urine.

39. The method of claim 37, wherein processing comprises at least one of:

(a) adjusting a time of incubation;

(b) adjusting a temperature;

(c) adjusting a pressure;

(d) adjusting solution pH;

(e) subjecting the samples to a nucleation event;

(f) subjecting the samples to ultrasound, shock waves, laser energy, or mechanical stimulation;

(g) adding a component; or

(h) adjusting an amount of the medium.

40. The method claim 37, wherein one or more of the samples further comprises one or more additional components.

41. The method of claim 40, wherein the additional component is a small molecule.

42. The method of claim 37, the array comprising at least 48 samples.

43. The method of claim 37, the array comprising at least 96 samples.

44. The method of claim 37, the array comprising at least 384 samples.

45. The method of claim 40, wherein one or more of the samples differs with respect to at least one of:

the identity or amount of one of the components;

the physical state of one of the components;

the identity or amount of the medium; or

the pH.

46. The method of claim 37 wherein the modification is the promotion of dissolution.

47. The method of claim 37, wherein the calculus is calcium phosphate, calcium carbonate, calcium pyrophosphate, calcium oxalate, a kidney stone, uric acid or a salt thereof.

48. The method of claim 37, wherein at least about 100 samples are screened per day.

49. The method of claim 37, wherein at least about 1,000 samples are screened per day.

50. The method of claim 37, wherein at least about 10,000 samples are screened per day.

51. A method for high throughput screening to identify potential inhibitors of at least one member of the set consisting of nucleation, crystal growth, agglomeration, aggregation, and tubular migration, adhesion, the method comprising:

dispensing a solution comprising supersaturated calcium oxalate into an array;

adding one or more components to a plurality of sites in the array;

incubating the array with the solution comprising supersaturated calcium oxalate and added plurality of components at a predetermined temperature; and

assessing changes in the solution comprising supersaturated calcium oxalate by at least one technique from the group consisting of measuring turbidity, microscopic morphometry, measuring total light output, image processing, particle counting, particle sizing, and birefringence.

52. The method of claim 51 wherein the solution comprising supersaturated calcium oxalate comprises urine.

53. The method of claim 52 wherein the urine is synthetic urine.

54. The method of claim 52 wherein the urine is human urine.

55. The method of claim 52 wherein the predetermined temperature is 37 °C.

56. The method of claim 52 wherein the array has at least 96 samples.

57. The method of claim 52 wherein the array has at least 384 samples.

58. The method of claim 52 wherein the array has at least 1536 samples.

59. The method of claim 52 wherein the array is implemented in one or more multiwell plates having wells selected from the group consisting of 24 wells, 48 wells, 96 wells, 384 wells, and 1536 wells.

60. The method of claim 59 wherein the multiwell plates have glass-bottomed or plastic-bottomed wells.

61. The method of claim 52 wherein the array is implemented by a collection of containers in at least one block.

62. The method of claim 52 wherein the plurality of compounds comprise calcium oxalate crystals.

63. The method of claim 52 wherein an absorbance at about 620 nm is measured for at least one sample in the array.

64. The method of claim 63 wherein a time course of the absorbance is measured.

65. The method of claim 63 further comprising detecting a decrease in the absorbance relative to a control sample to identify a corresponding compound or condition.

66. The method of claim 51 further comprising obtaining an image exhibiting birefringence in polarized light for at least one sample in the array.

67. The method of claim 66 wherein individual crystals in the at least one sample are imaged.

68. The method of claim 67 further comprising determining a digital representation of total light output.

69. The method of claim 67 further comprising determining the number of discrete light spots in an image thereby estimating the number of particles.

70. The method of claim 51 wherein crystal habit is determined.

71. The method of claim 70 wherein the habit is determined by mapping pixel positions and deriving borders.

72. The method of claim 67 further comprising discriminating between nucleation, growth and agglomeration of crystals.

73. The method of claim 66 further comprising obtaining an image of a glass-bottomed or plastic-bottomed multiwell plate in polarized light for at least one sample in the array implemented on the glass-bottomed or plastic-bottomed multiwell plate.

74. The method of claim 51 wherein the incubation is for a defined duration.

75. The method of claim 51 further comprising removing solution to leave behind crystals.

76. The method of claim 75 further comprising adding back fresh supersaturated calcium oxalate solution.

77. The method of claim 76 further comprising measuring crystal growth following adding back of fresh supersaturated calcium oxalate solution.

78. The method of claim 51 further comprising collecting and drying the crystals.

79. The method of claim 51 further comprising adding a defined amount of calcium oxalate crystal powder to each well.

80. The method of claim 79 further comprising estimating an number of calcium oxalate crystals added to each well with a hemocytometer.

81. The method of claim 67 further comprising determining a location and area of each crystal in a well in the multiwell plate.

82. The method of claim 81 further comprising repeating the location and area determination after a predetermined time to estimate crystal growth over the predetermined time.

83. The method of claim 82 further comprising averaging crystal growth over crystals in the well to obtain an average growth rate and standard deviation thereof.

84. The method of claim 67 further comprising shaking at a specified speed for a predetermined period.

85. The method of claim 84 further comprising reimaging to obtain a second size distribution for the crystals.

86. The method of claim 85 further comprising averaging crystal growth over crystals in the well to obtain an average growth rate and standard deviation thereof.

87. The method of claim 51 wherein at least one of the one or more compounds is a small molecule.

88. The method of claim 51 wherein at least one of the one or more compounds is a protein.

89. The method of claim 1, 37 or 51 wherein the component is selected from the group consisting of 4-bromomandelic acid, sucrose octaacetate, agaric acid, tetrahydrofuran-2,3,4,5-tetracarboxylic acid, atrolactic acid hemihydrate, methylene blue, lactobionic acid, benzilic acid, mandelic acid, lactobionic acid hemi-calcium salt, indomethacin, furosemide, hippuric acid, aromatic compounds, heteroaromatic compounds, quazalones, perazanones, uracils, statins (HMGco-reductase inhibitors), sulfated alcohols, alkylsulfides, ethers, polyethers, peptoids, sugars, and probenicid.

90. A method of treating nephrolithiasis in a human in need thereof, which method comprises administration to said human one or more compounds selected from the group consisting of 4-bromomandelic acid, sucrose octaacetate, agaric acid, tetrahydrofuran-2,3,4,5-tetracarboxylic acid, atrolactic acid hemihydrate, methylene blue, lactobionic acid, benzilic

acid, lactobionic acid hemi-calcium salt, indomethacin, and furosemide, or a pharmaceutically acceptable salt, solvate, hydrate, derivative, ester or prodrug thereof.

91. A method of managing hyperoxaluria in a human in need thereof, which method comprises administration to such human one or more compounds selected from the group consisting of 4-bromomandelic acid, sucrose octaacetate, agaric acid, tetrahydrofuran-2,3,4,5-tetracarboxylic acid, atrolactic acid hemihydrate, methylene blue, lactobionic acid, benzilic acid, lactobionic acid hemi-calcium salt, indomethacin, and furosemide, or a pharmaceutically acceptable salt, solvate, hydrate, derivative, ester or prodrug thereof.

92. The method of claim 1 whereby the identified condition is one of the natural parameters of human urine including:

osmolarity;

pH;

total ion concentration;

concentration of particular ions;

the ratio of the concentrations of said ions; and

the concentration of proteinaceous urine components that may modify calcium oxalate crystal formation, growth, agglomeration, adhesion to cell surfaces, or other relevant parameters.

93. The method of 92 wherein the ion is selected from the group consisting of calcium, sodium, potassium, oxalate, phosphate, citrate, chloride and magnesium.

94. The method of claim 92 wherein the urine components are Tamm-Horsfall protein or albumin.

95. The method of claim 92 wherein the identified condition can be used as a prognostic diagnostic to estimate lithogenic potential of a human patient.

96. The method of claim 95 wherein estimation of lithogenic potential of a human patient can be used as a clinical guideline for initiation of preventive therapy using available therapeutics.

97. A method for identifying conditions, compounds or compositions that promote dissolution of calcium oxalate crystals or calculi which comprises:

preparing an array of samples comprising calcium oxalate crystals or calculi and one or more components in urine;

processing the array;

analyzing the samples of the array to identify conditions or components that promote dissolution of the calcium oxalate crystals or calculi.

98. A method for diagnosing patients having an increased risk for lithogenicity which comprises:

processing seed crystals of calcium oxalate in urine from a patient;

analyzing the urine samples to determine the size, number and habit of any crystal or calculi.

99. The method of claim 98 wherein the processing comprises adjusting at least one of: time of incubation, temperature and pressure.

100. The method of claim 98 further comprising processing the urine in the presence of one or more components.

101. The method of claim 98 wherein an array of urine samples is processing, each sample in the array differing from the other by concentration or one or more components.

102. A method for identifying conditions, compounds or compositions for inhibiting nucleation or crystal growth which comprises:

preparing a supersaturated calcium oxalate solution;

seeding said supersaturated solution;

removing the supersaturated solution after crystals have formed; and

contacting the crystal with a second solution comprising one or more test components and analyzing the crystals after exposure to the second solution.